



IN THE UNITED STATES PATENTS AND TRADEMARK OFFICE

In re application of Kazuo Sakuma

Art Unit: 1651

Application No.: 10/066,224

Examiner: Ms. Ruth A. Davis

Filed: 02/01/2002

For: An antiviral therapeutic drug with therapeutic effects on syndromes Preventive and therapeutic agents for microbe-related syndromes including HIV

Remarks (reply to the Office Action)

Dear Ms. Ruth A. Davis:

In these remarks, the Applicant responds to the objection indicated by the examiner concerning the specification and argues the opinions. The Applicant ask the examiner to reconsider and examine further the specification and claims.

Below, I express my views in order on the issues indicated by the examiner in the detailed action of rejection and the reasons for objection and/or rejection, and ask you to reconsider them.

Election/restrictions of claims

1. Concerning the disposition of claims notified, since the Applicant modified the selected subjects and title in the specification, modifications were made to claim 1 and other claims as shown in the complete listing of all claims.

Specification

2. In response to the indication of the examiner, I wrote the summary in a single paragraph to avoid the legal phraseology of "said" in the claims.

3. In response to the indication of the examiner, the Applicant adds a simple explanation of Figures 23 and 24 in the attached specification.

Claim objection

4. Since I modified the content of claim 1 due to modification of the selected subject of the specification, I have not used the term "ATL," which was indicated to modify "ALT" to "ATL." And according to proper Markush language, by inserting "consisting" after "group", I modified the forms of all the claims to an official format.

According to the indication of the examiner, I also placed the official name before the abbreviation MRSA, put parentheses around the abbreviation. I have not used the term "Streptococcus" in the amended claims.

Concerning the improper form of a multiple dependent claim indicated in claim 25, since I withdrew claim 25, no action in response to the examiner is necessary.

Claim Rejections

5. That the specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention has been indicated.

6. Here, the applicant has been indicated that the claims concerning the preventive effects on the syndrome, therapeutic effects and invention are unclear.

Claims 7 and 8 have been deleted because they were indicated as being unclear.

Concerning the lack of sufficient antecedent basis in Line 1, "The preventative," of claim 1 and Line 3, "the microbes," of claim 3, since I offered claim 1 newly based on the modification of the subject of claims, I have used neither of the two terms. The same applies to "various syndromes possibly caused by" in claim 1.

Claim 11 was withdrawn and its right was confirmed in claim 5 of the claims.

Additionally, the examiner indicated that the standard antecedents for "HIV" in claim 26 are insufficient and it is unclear what is included or excluded in Claim 27: Scope of patent, but since I withdrew these claims from the claims of this specification, I have not responded to them.

7. The examiner requested us to specify the best form represented by the inventor for development of the object.

8 . Concerning the indication in item 8, I modified the subject of the specification to "An antiviral therapeutic drug with therapeutic effects on syndromes" to modify the specification to meet the enablement requirement.

The objects not described in the specification are contained in claims 1 to 12 and 25 to 27. Description of the preventive effect on HIV, etc. deviates from the enablement requirement. The working examples of Kabanoanatake are insufficient (including the previous item 7).---- The expression of the respective claims was changed and updated.

9.The applicant has been indicated that if it is described in prior documents more than 1 year before the date of application for patent in the USA, it will be declined.

10.The examiner indicated that Claims 1 to 12 and 25 to 27 were declined under 35USC.102(b) because they correspond to Sakuma and have no novelty. But since I constructed the structural difference in the objective of use and culture method of Kabanoanatake from the conventional technology by the present modification of claims, I assert that novelty is present in the claims.

11.The application of the Applicant was evaluated that it has no novelty or correspondence to JP09-191891 Sakuma, etc.

As concerns the indication concerning Claims 9 to 11, the product was invented by Sakuma, but it is insufficiently effective to be applied for in the USPTO. Several errors in translation have been corrected. As described in the specification, monolignin and pseudohumic acid are described as the active ingredients (line 13-16 on Page 3). Their molecular weight is less than 450,000. Moreover, the presence of methoxyl groups in humic acid is usually 0.5% to 1%, but in this invention, pseudohumic acid, they are 2.5% to 3.5%.

(The proportion of methoxyl groups in the dry precipitate after acidifying the extracted substance to pH 2.5 rises to 1-2% and 5-7%, respectively.)

In this application, this substance is for practical reasons called pseudohumic acid or polyphenol complex due to the material properties of the main component of the active ingredients. In the specification, Kabanoanatake extract was specified as the primary processed matter, substances with molecular weight of less than 450,000 were designated as this substance for reasons of clarification. Figures 1b to 4b and Figures 6-A2 and 6-B2 were added since they were required. Figures 7b to 9b and 10b have also been added for clarification.

Difference from the prior invention

Therefore, the dose of this invention is less than 50% of my prior invention, that I, Kazuo Sakuma, described in the prior invention as indicated by the examiner. For patients with weakened swallowing ability and weakened organ functions, small doses give great relief. In addition, in comparison with the prior invention of Sakuma as indicated by the examiner, this invention has better absorability into the human body and better solubility, which is far superior to substances made by conventional techniques. In the general explanation of Kabanoanatake, the data on the substance

made using the primary processed matter described in the claims of this application was used because this substance is the invention of the applicant himself. The actual drug efficacy of this drug is extremely high. When the ingredients with drug efficacy, particularly antiviral ingredients, are examined in detail, Kabanoanatake extract has a molecular weight of less than 450,000. The details are as shown below.

Concerning the molecular weight of 450,000 of the Kabanoanatake extract I will briefly show a concrete example. The ingredients extracted from Kabanoanatake can be fractionated into various fractions with different molecular weights by techniques such as gel filtration. In the processes of extraction and fractionation, the natural sclerotium of Kabanoanatake is extracted with hot water (water: solid = 6:1), and 100 mg of dry powder obtained by freeze-drying the extract is dissolved in 10 ml of distilled water. This solution is centrifuged to remove debris, purified, applied to a column and fractionated into its respective fractions by gel filtration. The molecular weights of the respective fractions, shown in Table A, were obtained using these procedures. In the gel filtration process, which generated the data shown in Table A below, blue dextran (molecular weight 1,000,000), ferritin (molecular weight 450,000), myoglobin (molecular weight 17,800) and DNP-L-alanine (molecular weight 255) were used as the molecular weight markers. Kabanoanatake exhibits similar efficacy in natural form and as artificially cultured hyphae (cultured in solid or liquid media). In addition to hot water, the active ingredients can be extracted with ethanol or other solvents.

Molecular weight and anti-HIV activity of Kabanoanatake

The anti-AIDS virus activity in relation to the molecular weights of the respective fractions obtained by gel filtration shown in the Table A was examined. If uninfected cells (Molt 4/c 18 cells) of healthy volunteers and AIDS virus-infected cells (Molt 4/c HIV III B) are cocultured, the uninfected cells adhere to the infected cells and fuse to form giant cells. This is a form of proliferation of the AIDS virus. It was found that, when aqueous Kabanoanatake extract is added to this coculture system, the formation of giant cells is markedly inhibited.

Briefly, the infected cells and the uninfected cells were mixed at a ratio of 1:1 (1×10^6 : 1×10^6) and diluted. The components of the respective fractions of the Kabanoanatake extract were added to this diluted coculture system at a concentration of 10 $\mu\text{l}/\text{ml}$, the mixture was incubated for 24 hours, and then the diameter of the cells was determined using a multisizer. Cells more than 20 μm in diameter were categorized as giant cells.

The incidence of the cells was counted, and the value of 100 – incidence (%) was regarded as the inhibition rate (%). The results are shown in the column of inhibition rate (%) in Table A.

Table A

<u>Activity corresponding to the molecular weight of Kabanoanatake extract</u>	<u>Fraction (Fr) No.</u>	<u>Molecular weight</u>	<u>Inhibition rate (%) of HIV infection</u>
	25 (Blue dextran)	More than 1,000,000	0
28		950,000	0
29		760,000	0
30		600,000	0
31 (Ferritin)		450,000	55.05
32		360,000	58.31
33		280,000	61.43
34		220,000	62.35
35		170,000	67.15
36		130,000	71.30
37		100,000	78.25
38		80,000	81.99
39		62,000	100.00
40		50,000	80.49
41		40,000	95.29
42		30,000	89.30
43		24,000	85.60
44 (Myoglobin)		17,800	61.38
45		14,000	67.99
46		11,000	83.14
47		10,000	65.35
48		8,000	68.16
49		6,600	71.33
50		5,400	50.37
51		4,500	50.25
52		3,700	47.91

55	2,000	86.39
58	1,200	51.34
65	290	47.72
66 (Alanine)	255	46.18
68	190	45.87

Explanation of the inhibition rate shown in Table A

As concerns the inhibition rate shown in Table A, anti-AIDS activity is observed extensively in the respective fractions whose molecular weight is less than 450,000 (fraction 31). For molecular weights ranging from 220,000 (fraction 34) to 10,000 (fraction 47), an inhibition rate of 60% to 100% was achieved. At the molecular weights of 62,000 (fraction 39), 50,000 (fraction 40), 40,000 (fraction 41) and 30,000 (fraction 42), particularly, the inhibition rates were 100%, 80.49%, 95.29% and 89.30%, respectively, showing very high efficacy. The inhibition rate was 83.14% and 65.35% at the molecular weights of 11,000 (fraction 46) and 10,000 (fraction 47) respectively. It is noteworthy that the AIDS inhibition rate is high as 68.16% and 86.39% even at molecular weights of less than 10,000, such as 8,000 (fraction 48) and 2,000 (fraction 55), respectively. Achievement of an AIDS inhibition rate of 45.87% at a low molecular weight of 190 (fraction 68) indicates that Kabanoanatake extract is active even at low molecular weights. It is therefore a characteristic of this product that, unlike the efficacy in a narrow molecular weight range usually observed in other substances, this invention, Kabanoanatake extract, shows efficacy at molecular weights of less than 450,000 and is widely distributed in an undefined form which cannot particularly be limited.

Utilization of active ingredients and significance of this drug to treat viruses

From this point of view, as concerns this invention, the Kabanoanatake extract, the active ingredients corresponding to the respective molecular weights are not taken out *en masse*, but it can be said that, to prevent drug resistance being acquired by viruses, it would be more logical to use the fractions with molecular weights of less than 450,000 comprehensively. It is said that AZT causes resistance within a few days of administration. Actually, AIDS patients treated with AZT for 3 months acquire resistance. No drug resistance was, however, acquired even after administration to the applicant himself for one and a half years. When its functions were examined, it was

found that this substance inhibits the reverse transcriptases released from retroviruses and inhibits proteases. As explained thereafter, since Kabanoanatake extract contains a spectrum of various components with different molecular weights, has antibacterial effects and has Superoxide dismutase (SOD)-like activity of 11 units, it is effective against various diseases and symptoms, such as those induced by retroviruses, including AIDS virus, and other viruses. Since Kabanoanatake extract heals diabetes mellitus through its SOD-like effect and inhibits degenerative processes such as cancer and aging, it has been accepted as a subject of presentations at academic conferences. Next, we will describe various basic experiments leading to the confirmation of effectiveness of this invention.

Combustion experiment for identification of ash

As a basic experiment (1) to investigate this invention, the composition of ash of Kabanoanatake was examined. To be specific, an experiment was conducted by combusting dried natural Kabanoanatake. When dried Kabanoanatake is combusted, it smokes continuously until fully combusted. The residual ash is soft, has a light greenish-blue color and is hygroscopic. The weight of this ash averaged about 13% of the dry weight of the starting material, Kabanoanatake. More than 50% (corresponding to about 7% of the starting material) comprised potassium and sodium. In comparison with common mushrooms (for example, the shiitake mushroom), since 4.7% of the dry weight of the fruiting body of shiitake mushroom is ash, it was confirmed that Kabanoanatake contains a much larger amount of ash than observed in common mushrooms. The average composition of ash, confirmed by multiple experiments, is shown in Table B below.

Table B

Cation composition (weight %) and anion composition (weight %) Mn₂O₃: 1.25; P₂O₃: 8.79; CuO: 0.004; SO₄: 0.05; ZnO: 0.056; CO₂: 40.85; Na₂O: 9.60; K₂O: 42.70; MgO: 2.39; CaO: 1.92; Al₂O₃: 0.16; Fe₂O₃: 0.16; SiO₂: 1.80; and SO₄: 5.80.

As observed in the above Table B, the ash remaining after combustion of Kabanoanatake contains K₂O at 42.7% and Na₂O at 9.6%, meaning that more than 50% of the ash is potassium and sodium. The experiment was conducted using natural Kabanoanatake, but since artificially cultured Kabanoanatake (in solid and liquid media) also tends to contain sodium and potassium in the composition of its ash, it should be possible to remove them if the contents are unnecessarily high.

It was found from a great number of experiments that this Na increases anti-viral effects when it binds to the pseudohumic acid in Kabanoanatake. That is, common humic acid (containing 1% to 2% methoxyl group when precipitated in acid) shows no efficacy. The active ingredient, mainly contained in this invented substance with a molecular weight of less than 450,000, so-called pseudohumic acid, contains methoxyl groups at 5% to 7% in precipitates obtained with hydrochloric acid at pH 2.5 to 3. If a substance contains a large number of methoxyl groups, antiviral effects will appear. Typical substances containing minimum methoxyl groups include guaiacol, which has a benzene ring, and 2-6 dimethoxylphenol.

Analysis of the composition of extract

Next, as a basic experiment (2), the chemical composition of the hot water extract of Kabanoanatake was analyzed. When this extract was concentrated and the chemical composition of the ash contained in it was analyzed, it was found that the whole ash comprised 63.2% potassium, 13.1% sodium and 1.83% magnesium. As observed in the composition of ash obtained after combustion of Kabanoanatake, it was found that high levels of potassium and sodium compounds were present in the hot water extract. These results, shown in Table C, show the binding of Na to pseudohumic acid mentioned previously, and it is obvious that, to obtain active ingredients of high purity, the sodium compounds contained in the Kabanoanatake extract should be retained. As observed in the combustion experiment, natural Kabanoanatake and artificially cultured Kabanoanatake are equivalent.

Table C

Name of oxide Weight% of the whole ash

Na₂O: 13.10; K₂O: 63.20; Mn₂O₃: 1.05; MgO: 1.83; CaO and trace Fe₂O₃: 0.39; and SiO₂: 1.66.

Next, an example of the general processes of manufacturing the drug of this invention (related to highly pure drinkable preparations and foods) is shown inclusively using water.

(a) Extraction with hot water: To dried and finely pulverized Kabanoanatake, add water (more than six times the dry solid weight) to prepare a hot water extract. Boil the water containing fine granules of Kabanoanatake at about 90 to 100 °C for about 1 hour. If the active ingredient cannot be extracted sufficiently the first time, squeeze a cloth bag

containing the first extract obtained by boiling, and then add an appropriate volume of water to the residue after extraction of Kabanoanatake again and boil at 50 to 100 °C for about 1 hour. Qualitatively, it is desirable to boil at 50 to 60 °C at which no methoxyl groups are reduced. After squeezing again, mix the first and second extracts.

(b) Gel filtration: Fractionate the active ingredients with a molecular weight of less than 450,000 using a molecular sieve technique (for example, by gel filtration) or other techniques for removal of low molecular weight substances.

(c) Drying: Dry the solution (containing the active ingredients) obtained in the previous item (b) by freeze-drying, etc.

(d) Preparation of liquid formulation: Dissolve the dried material (powder) obtained in the previous process (c) as it is or with excipients in isotonic sodium chloride solution at the specified solid ratio to prepare a liquid formulation (injection, etc.). In addition, a powder of this dried material can be incorporated as it is into various drink preparations (beverages) and the raw materials of foods, such as skimmed milk, soup or fluid diet ingredients.

(e) Storage: Transfer the liquid formulation prepared into containers, for example, ampoules or plastic bags (for instillation) for storage after sterilization. It is desirable to transfer a portion of liquid formulation for one-time use and to store it in a refrigerator. Alternatively, it is possible to store the dried material in powder form at the stage of drying process (c) or to encapsulate it in a microcapsule and use it after dissolution, if necessary. Drink preparations or foods should be packed in containers such as bottles and packs in accordance with an ordinary method.

The processes reviewed above should be scaled up or down according to the scale of working but are not limited to this, and other processes can be conducted, if necessary. In addition to water, the active ingredients of Kabanoanatake can be extracted using, for example, PBS solutions, butanol, ethyl acetate, acetone, alcohol, alcohol + water or chloroform. Since LPSs (lipopolysaccharides) that induce macrophage activity are contained in Kabanoanatake, however, extraction with water (hot water), by which it is easy to obtain LPSs (lipopolysaccharides), is desirable. It is of course necessary to consider reduction of LPS depending on the concentrations.

The manufacturing processes

Next, we will give a concrete example of the manufacturing processes of high purity active ingredients of this invention reviewed in the previous item. The respective processes (a) to (f) correspond to those previously reviewed. In process (a), moreover, natural Kabanoanatake as well as Kabanoanatake cultured in solid sawdust (of birch, particularly) medium and that cultured in a liquid medium were examined for reference.

- (a) Two hundred (200) g of natural Kabanoanatake was boiled in 1200 ml of water. From this solution, a total of 60.1 g of dry material, a shiny blackish brown, was observed. This dry material had a bitter, woodlike odor. From 2.3 kg of undried material cultured in a solid sawdust medium, on the other hand, a total of 30 g of dry material was obtained. It had a bitter savory taste. From 300 ml of cultured liquid media, 2.3 g of the dried hot water extract of hyphae and 1.03 g of the dry weight of the solution were measured, and a total of 1.53 g was obtained from these liquid cultures.
- (b) The hot water extract of natural Kabanoanatake was treated with acid and the precipitate collected. From 60.1 g of the dried extract of natural Kabanoanatake, 30 g of precipitate was obtained.
- (c) From 30 g of the precipitate described above, acid and the impurities K₂O were removed by gel filtration.
- (d) The solution containing active ingredients, obtained by removing the acid and impurities, was dried by freeze-drying, and 13.5 g of dry powder of natural Kabanoanatake was obtained. In addition, 2.9 g (dry weight) of the purified material of molecular weight of less than 450,000 obtained by gel filtration (S300) was obtained. As explained later, various physiological activities, including antiviral activity such as anti-AIDS virus activity and anti-influenza virus activity, were widely observed in these various fractions.
- (e) Dissolve the dry powder of active ingredients in distilled water or isotonic sodium chloride solution to make solutions containing 0.01% to 99% (weight), actually 0.05% to 50%, appropriately 0.1% to 5% and ideally 0.7% to 3%.
- (f) Encapsulate the liquid formulation for injection, sterilize, and store the capsule. If this formulation is used for instillation, put the liquid formulation into plastic bags for

instillation, sterilize and store the plastic bags. This invention, the Kabanoanatake extract, can be used in various forms as tablets, granules, capsules and drink formulations (beverages) or as a food containing active ingredients of Kabanoanatake for oral administration.

Concerning amendments to drawing figures

To eliminate any confusion between Kazuo Sakuma's prior art substance and the new invention submitted in this application to the United States, the prior art invention is referred to herein as the "primary processed material," and said new invention, with a molecular weight less than 450,000, is referred to as the "present invention."

Figures 1b, 2b, 3b, 4b, 6A-2, 6B-2, 7b, 8b, 9b, 10b, 25, and 26 were added to show the clear superiority of the present invention. For the same reason, the term "primary processed material" was added to Figures 1, 2, 7 and 9 in order to indicate that these figures are for the primary processed material.

Additionally, grammatical and other language errors were corrected in the following ways.

Fig. 2

Inhibition effects of anti-HIV agents on HIV production by PHA-stimulated peripheral blood mononuclear cells that was made to be newly infected.

→ Inhibition effects of anti-HIV agents of the primary processed matter on HIV production by PHA-stimulated peripheral blood mononuclear cells that were made to be newly infected.

Fig.6 B-1

The effects of addition of Kabanoanatake in various incubation time after target cells pretreatment with anti-HIV agents for approximately one hour

→ The effects of addition of Kabanoanatake in various incubation times after target cells pretreatment with anti-HIV agents for approximately one hour

Fig.10

Annotation) In also a blood test after three months for the same patient, TCID value was excellent (zero).

→ Annotation) Also, in a blood test after three months for the same patient, the TCID value was excellent (zero).

Fig.11

Ant-HIV activity →Anti-HIV activity

The well number below 1 means that perfect inhibition effects on HIV is not obtainable.

→A well number below 1 indicates that perfect inhibition effects on HIV is not obtainable.

Fig.13

number of wells showed perfect inhibition

→number of wells showing perfect inhibition

Culture temperature of diurnal time →Diurnal culture temperature

Culture temperature of nighttime →night culture temperature

Fig.14

Culture temperature of diurnal time →Diurnal culture temperature

culture temperature of nighttime →night culture temperature

Fig.15

added lignin ,under extreme conditions →with lignin, under extreme conditions

Fig.16

added lignin, under extreme conditions →with lignin, under extreme conditions

Fig.17

The control group →The control groups

Fig.18

④AIW-27 and 03glignin area →④AIW-27 and 03g lignin area

The control group →The control groups

Fig.19

added lignin, restricting the infiltration of oxygen →with lignin, restricting the infiltration of oxygen

The control group →The control groups

Fig.20

added lignin, under extreme conditions →with lignin, under extreme conditions
The control group →The control groups

Fig.23

AIW-4, added lignin substances →AIW-4, with lignin substances

Conclusion

When the fractions of Kabanoanatake of the respective molecular weights were obtained by column chromatography and tested for their anti-AIDS virus activity, as shown above, it was concluded that the active ingredients applied for are present with molecular weights of less than 450,000, and the main substance is pseudohumic acid, but since then, this description has been corrected and improved. The substance of the molecular weight of less than 450,000 derived from Kabanoanatake has a new possibility and a structural composition of substance, shows very few adverse reactions, and is beneficial to the diseased body. In addition, it was found that this substance blocks the route of approach of viruses, simultaneously inhibits reverse transcription and protease and is effective against influenza virus type A (H1N1), type B, and the H₃N₂ (Hong Kong) type.

The present invention is a new development based on the pharmaceutical concept of using a single drug for a variety of effects. It couples antiviral activity with therapeutic effects against a wide range of syndromes. Therefore, the necessary and novel techniques for production and harvest thereof are fully disclosed herein. Although a portion of the effects do resemble those of prior art techniques, the present invention constitutes a truly innovative leap in terms of pharmaceutical effects and underlying concepts of drug discovery. In addition, a variety of new content has also been presented herein, including the relationship between carcinogenetic promotion and chemical compounds found in the present invention, the powerful effects of humic acid, pseudo-humic acid and sodium compounds, the relationship between anti-AIDS effects and molecular weight, as well as anti-fat agglutination and physical strength reinforcement effects.

None of these disclosures can be found in prior art or literature, so they have novelty value.

As indicated, since this application was originally badly set out, we have modified and corrected it. In particular, we have emphasized the benefits of this invention in this application by differentiating it from the primary processed substance extracted from

the Kabanoanatake fungus. We submit this application together with the notification for modification of specification. We hope that you will continue this discussion and decide to grant us a patent.

Kazuo Sakuma

By *Kazuo Sakuma*

2119-1, Kaminayoro, Shimokawa-chou,
Kamikawa-gun, Hokkaidou, 098-1216
JAPAN